The Action on Bacteria of Electrical Discharges of High Potential and Rapid Frequency.

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In the course of an investigation into the action of "high frequency" electrical discharges as they are used in the practice of medicine, we have inquired more particularly into the action of such discharges on bacteria exposed to their influence under various experimental conditions.

I. General Procedure in Experiments, and Electrical Apparatus Used.

The bacteria used for the purposes of our experiments have included the following species:—

B. anthracis, *B. diphtheriæ*, *B. typhosus*, *B. coli communis*, *B. dysenteriæ* (Shiga), *B. pyocyaneus*, and *Micrococcus pyogenes aureus*, together with some saprophytic species, *B. megaterium*, *B. prodigiosus*, and *Micrococcus agilis*.

In our experiments the bacteria have been exposed to the action of the discharges under the following conditions:—

(1) The discharges have been sprayed on to the surface of distilled water, tap water, and normal saline solution in which the bacteria were suspended; and with these circumstances the current has been discharged in different atmospheres above the bacterial emulsion, experiments having been carried out in common air, hydrogen, nitrogen, carbon dioxide and carbon monoxide.

(2) The discharges have been sprayed directly on to the surface of cultures of the several bacteria whilst growing on nutrient-agar and various other solid culture media.

(3) The discharges have been sprayed directly on to the surface of thin films of the bacteria spread out on small slabs of plaster of paris.

And, in addition, we have tested the action of rapidly oscillating electrical currents of high potential when passed directly through bacterial emulsions without interruption by discharge above the surface of the fluid. The experimental conditions included under (2) and (3) above were soon found to be unsatisfactory, inasmuch as a high degree of heat was quickly concentrated in the solid material on which the bacteria were exposed, and it

was impossible to appreciate what other kind of influence, if any, the electrical discharge might exert. In what follows we shall refer, therefore, only to the results of experiments carried out under the conditions defined in (1), referring incidentally to the question of the possible influence of the passage through the emulsion of rapidly oscillating currents of high potential without interruption by discharge.

The Electrical Apparatus Used.

In some experiments the oscillatory discharge of high potential obtained at the terminals of the secondary coil of the Ruhmkoff apparatus was transformed by means of a spark-gap, Leyden jars, and resonating Tesla coil into an oscillatory discharge of high potential and extremely rapid frequency.

In the majority of our experiments, however, the apparatus was of different arrangement. The current obtained from the electric lighting main was transformed into an oscillatory current of very high potential by means of an alternator (connected with a transformer) which was driven by a motor. Wires from the alternator were connected with the terminals of a spark-gap and a large condenser immersed in oil. With this form of apparatus the transformation of the current is closely analogous with that occurring when the first mentioned form of apparatus is used.

A third set of experiments were carried out with a Ruhmkoff coil which gave a 12-inch spark; these last experiments being for the purpose of comparing our other results with those which might be obtained by the use of electrical discharges of less rapid frequency, but such as were known to produce under certain circumstances distinct physiological reactions.

In our earlier experiments the action of the discharges was tested by spraying the current from a platinum wire brush on to the surface of either distilled water or normal saline solution containing various bacteria.

The emulsion of bacteria in the test-tube was exposed to the action of discharges sprayed on to them through common air in one or other of the following ways:—

(*a*) The current was discharged from the points of a platinum brush formed by a disc of platinum, 1·5 cm. in diameter, to which were attached nine short lengths of platinum wire; the disc was suspended with its surface parallel to the surface of the bacterial emulsion, the ends of the wire brush being between 2 and 5 cm. above the fluid. In one or two experiments a different form of brush, made by twisting together some lengths of platinum wire, was used, but the resulting discharge was very much less energetic than when the other form of brush was used.

The current being discharged over the emulsion from the brush, it was conducted away by means of a short length of platinum wire which was sealed through the bottom of the test-tube and dipped into a layer of mercury at the bottom of a beaker of water in which the test-tube was suspended for cooling purposes, the level of the water in the beaker being kept well above the level of the bacterial emulsion in the test-tube. From the layer of mercury in the beaker an "earth wire" passed to a gas supply pipe.

In some of the experiments carried out with this arrangement of apparatus the cooling beaker was not used, and the electrical current was allowed to escape into the air as a brush discharge from the platinum wire which passed through the bottom of the test-tube.

(*b*) In another set of experiments there was a layer of mercury at the bottom of the bacterial emulsion in the test-tube, but no wire passing from the tube. In these experiments the results were not satisfactory, for the current appeared to diffuse in various directions after its discharge, and apparently but a small proportion of it passed through the emulsion.

(*c*) In another type of tube which was used, a cap of tin foil encapsuling the lower end of the tube was substituted for the length of platinum wire passing through the bottom of the tube; a few turns of copper wire were wound round this metal cap, and the efferent end of the wire was attached to a gas pipe. This type of tube also was found to give unsatisfactory results; when using it the current seemed to discharge itself chiefly towards the side of the glass tube at the upper level of the emulsion and then to pass down the side of the tube to the metal cap without passing through the emulsion itself.

In the experiments detailed in what follows it is to be understood that the arrangement for conducting the current after its discharge above the emulsion was that described under (*a*), and, unless the contrary is stated, the platinum disc brush was used. The arrangements described under (*b*) and (*c*) are mentioned for the reason that the apparently different course of the current subsequent to discharge which was observed with their use served to confirm our opinion that with the use of arrangement (*a*) the current after its discharge did actually pass directly through the emulsion.

For the course of these experiments it seemed probable that the bacterial emulsion was subjected to the influence of two factors: first there was the action of the discharge on the surface from the platinum brush, and then there was the action of such part of the current, if any, which subsequently passed through the fluid.

And as it appeared to us that the passage of a maximum portion of the

current through the emulsion was obtained with the first described arrangement (*a*), all the crucial experiments detailed in this paper were carried out with the kind of tube described, fresh distilled water being usually used for making the emulsion. Before proceeding it will be as well to give the reasons for our assumption that with this type of tube the current when discharged on to the surface is subsequently conducted directly through the fluid.

(1) When using the type of tube described the visible direction of the discharge is directly on to the surface of the fluid, and not towards the sides of the tube, as frequently is the case when the other arrangements are used.

(2) On raising the platinum wire which passes through the bottom of the tube from the mercury in which it is usually immersed a brilliant discharge breaks from its lower end.

(3) If the wire which passes from the mercury in the beaker to the gas pipe is removed for about half an inch from the pipe a brilliant discharge across the gap breaks.

(4) If tap water was used instead of distilled water, or even if distilled water which had been allowed to stand exposed to the air for a few days was used instead of freshly distilled water, a considerable portion of the current, instead of passing by the direct path through the fluid, appeared to pass from the edge of the platinum disc to the side of the test-tube, and thence through the water in the containing-beaker to the earth wire; whereas, when freshly distilled water was used, there was no indication of discharge towards the side of the tube, but the discharged current appeared to impinge directly on to the surface of the fluid, and apparently passed through it to the wire at the bottom of the tube.

(5) Unless special means were adopted for keeping the tube cool, a considerable and rapid rise in the temperature of the emulsion occurred.

It is true, however, that an alternative route was possible theoretically; that is to say, it is conceivable that after its discharge the current might be deflected at a right angle along the surface of the fluid, and then pass down between the side of the tube and the contained emulsion to the wire at the bottom of the tube. But there was no indication that this route was followed, and what we actually observed appeared to us to justify the conclusion at which we arrived as to the direct passage of the current through the emulsion.

In testing the effect of the discharges upon bacteria, the following was the procedure followed:—The bacterial emulsion was prepared by rubbing up two loopfuls of growth, generally taken from a 48-hours-old culture on agar, with 10 c.c. of distilled water. When a uniform emulsion had been made, a loopful of it was streaked out on each of two sloped nutrient agar tubes.

The emulsion was then subjected to the action of the discharge for the required time, at the end of which two agar tubes were each inoculated with a loopful of the treated emulsion. All the agar tubes were then incubated side by side at a temperature suitable to the growth of the particular organism under experiment, and any differences in growth on the experimental and control tubes were noted from time to time. At the conclusion of an experiment any necessary chemical examination of the emulsion was made immediately after the agar tubes had been inoculated.

II.—The Germicidal Action of the Discharge when Sprayed through an Atmosphere of Common Air.

Preliminary experiments, in which the bacterial emulsions contained in tubes of the type described under (*a*) were exposed to the action of the discharge of oscillatory currents of high potential and rapid frequency, sprayed on to the surface of the fluid through an atmosphere of common air, resulted in rapid destruction of the organisms. And we found that the discharge of the current on the surface of the emulsion was followed by the development of a considerable degree of acidity in the fluid, due chiefly, at any rate, to the presence of nitrous and nitric acids in solution.

Tables I and II give the results obtained in two sets of such experiments, in all of which precaution was taken to keep the bacterial emulsion at a temperature which could not by itself possibly affect the bacteria unfavourably, being below 30° C. in every case.

In the experiments detailed in Table I the current was transformed from the electric lighting main; the experiments of Table II were carried out with a powerful induction coil which gave a 12-inch spark, and the platinum brush was connected up with one terminal of the secondary coil, whilst the other terminal was connected with the layer of mercury at the bottom of the beaker in which the tube containing the bacterial emulsion was suspended. In every case, except in the experiments of Series 4 and 5 of Table II, the ends of the platinum brush were 2·5 cm. above the surface of the emulsion.

From the results given it will be seen that, except for the two sporing species, *B. anthracis* and *B. megaterium*, the bacteria tested were destroyed after comparatively short exposure to the discharge, when the ends of the brush were suspended at a distance of 2·5 cm. above the surface of the fluid, whilst in the few experiments included in Series 4 and 5 of Table II, when the brush was suspended 5 cm. above the surface of the fluid, a non-sporing organism, *B. coli communis*, escaped complete destruction, although some inhibition of subsequent growth was observed. In experiments with *B. anthracis* our results were not quite consistent; in the experiment of

Series 1, on Table I, this bacillus survived exposure to the discharge for 15 minutes, with a development of acidity in the emulsion equivalent to 0·19 per cent. of nitric acid, whilst in the experiment of Series 2 of the same table the bacillus was destroyed after exposure to the discharge for only 10 minutes, with resulting acidity of the emulsion equivalent to 0·14 per cent. of nitric acid. But an occasional inconsistency of results is only what may be expected in experiments of this kind, in which the primary factor is a form of electrical force which cannot be regulated with accuracy, and does not affect the main points at issue.

Table I.

Species.	Duration of exposure to the discharge.	Current at 100 volts supplied to the motor driving alternator.	Acidity (calculated as nitric acid per c.c.) of bacterial emulsion at end of experiment.	Percentage of acid in emulsion after experiment (calculated as nitric acid).	Result of experiment.
Distance of Platinum Brush above Surface of Fluid, 2·5 cm.					
Series 1.	mins.	ampères.	gramme.		
B. anthracis......	15	7	0·0019	0·19	Some growth, but marked inhibition
B. typhosus	15	7	0·0024	0·24	0
B. dysenteriæ ...	15	7	0·0023	0·23	0
B. coli communis	15	7	0·0021	0·21	0
B. pyocyaneus ...	15	7	0·0023	0·23	0
M. agilis	15	7	0·002	0·2	0
Series 2.					
B. anthracis......	10	6·75	0·0014	0·14	0
B. typhosus	10	6·75	0·0013	0·13	0
B. dysenteriæ ...	10	6·75	0·0014	0·14	0
B. coli communis	10	6·75	0·0016	0·16	0
B. pyocyaneus ...	10	6·75	0·0014	0·14	0
M. agilis	10	6·75	0·0014	0·14	0
Series 3.					
B. anthracis......	10	6	0·0008	0·08	+
B. typhosus	10	6	0·0011	0·11	0
B. dysenteriæ ...	10	6	0·0008	0·08	0
B. coli communis	10	6	0·0012	0·12	0
B. pyocyaneus ...	7·5	9	0·0009	0·09	0
M. agilis	15	9	0·0025	0·25	0
Series 4.					
B. typhosus	7	5	—	—	0
B. coli communis	7	5	—	—	0
B. pyocyaneus ...	7	5	—	—	0
B. megaterium ...	7	5	—	—	Some growth, but marked inhibition
B. prodigiosus ...	7	5	—	—	0

Table II.

Species.	Duration of exposure to the discharge.	Current and voltage (primary coil).	Acidity (calculated as nitric acid) of bacterial emulsion at end of experiment.	Percentage of acid in emulsion at end of experiment (calculated as nitric acid).	Result of experiment.
Distance of Platinum Brush above Surface of Fluid, 2·5 cm.					
Series 1.	mins.		gramme.		
B. anthracis	20	4 ampères 74 volts	0·0004	0·04	+
B. typhosus	20	"	0·0005	0·05	0
B. dysenteriæ ...	20	"	0·0001	0·01	0
B. coli communis	20	"	0·0009	0·09	0
B. pyocyaneus ...	20	"	0·0004	0·04	0
M. agilis	20	"	0·0009	0·09	0
Series 2.					
B. anthracis	15	6 ampères 80 volts	0·0005	0·05	+
B. typhosus	15	"	0·0006	0·06	0
B. dysenteriæ ...	15	"	0·0007	0·07	0
B. coli communis	15	"	0·0004	0·04	0
B. pyocyaneus ...	15	"	0·0005	0·05	0
M. agilis	15	"	0·001	0·1	0
Series 3.					
B. anthracis	10	6 ampères 84 volts	0·0005	0·05	Some growth, but marked inhibition
B. typhosus	10	"	—	—	0
B. dysenteriæ ...	10	"	—	—	0
B. coli communis	10	"	0·0008	0·08	0
B. pyocyaneus ...	10	"	0·0006	0·06	0
M. agilis	10	"	—	—	0
Distance of Platinum Brush above Surface of Fluid, 5 cm.					
Series 4.					
B. coli communis	10	6 ampères 80 volts	—	—	Some growth
B. pyocyaneus ...	12	"	—	—	0
" ...	10	"	—	—	0
M. agilis	6	"	—	—	0
Series 5.					
B. coli communis	5	6 ampères 80 volts	—	—	Some growth
B. pyocyaneus ...	5	"	—	—	0
" ...	5	"	—	—	0
Distance of Platinum Brush above Surface of Fluid, 2·5 cm.					
Series 6.					
B. anthracis	10	5·5 ampères 70 volts	—	—	+
B. typhosus	10	"	—	—	0
B. coli communis	10	"	—	—	0
B. pyocyaneus ...	10	"	—	—	0
B. prodigiosus ...	7	"	—	—	0

Having thus obtained definite evidence of the germicidal effect of the electrical discharge under the given experimental conditions, we had next to consider the respective influence of several possible factors in the production of the results which we had observed.

These possible factors, as they presented themselves to us, may be enumerated as follows:—

(1) What may be termed the specific physical action of the current, or of its discharge, on the bacteria in the emulsion;

(2) The action of light rays resulting from the discharge;

(3) The action of heat rays resulting from the discharge; and

(4) The action of certain chemical substances which are formed in the air as the result of the discharge, and are then taken up in solution by the water in which the bacteria are suspended.

III.—As to the Germicidal Action, if any, of the Electrical Current, or of its Discharge.

We met with considerable difficulties in our endeavour to determine what part, if any, in the production of the germicidal effects which had been obtained in the previously mentioned experiments could be attributed to the action on the bacteria of the electric current itself, as apart from secondary factors depending upon the discharge—the action of heat and light rays and of chemical substances formed in the neighbourhood of the emulsion and taken up by it. For, whilst it was easy to eliminate experimentally any injurious action which might be exerted directly by the heat rays and to test by itself the possible action of the light rays, we found the greatest difficulty in devising means whereby the bacteria could be subjected to the action of the electric current without at the same time introducing the possibility of fallacy arising from chemical decomposition with the formation of germicidal substances.

We first tested the action of the oscillatory current when passed directly through the bacterial emulsion without interruption by discharge above the surface. In these experiments a flat platinum disc, which was immersed just below the surface of the emulsion, was substituted for the platinum brush. In some experiments the emulsion was contained in a test-tube, with arrangement for conducting the current away as described under (*a*) above, but in the majority of the experiments there was the addition of a layer of mercury in the tube under the emulsion and covering the projecting upper end of the wire which was sealed through the bottom of the tube.

The latter modification appeared to be the more suitable for testing the full action of the current on the bacterial emulsion, and we found that when

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efficient means were adopted for keeping the temperature of the emulsion below the point at which the germicidal effect of heat would come into play, and when there was no great amount of "sparking" from the wire to the platinum disc, the mere passage of the current through the emulsion for periods of time corresponding to those prevailing in the previous experiments had no obvious effect on the bacteria. In certain experiments of this kind, in which the bacteria were destroyed, the emulsion was found to contain at the end of the experiment definite quantities of chemical germicidal substances which had been formed by excessive sparking just above the level of the emulsion from the wire which was connected with the immersed platinum disc.

If special precautions for keeping the emulsion cool were not taken, the heating effect of the passage of the current through the emulsion was very marked in these experiments with the immersed disc, the temperature rising rapidly to 55° C. or higher.

The following experiments with somewhat prolonged passage of the current through the emulsion may be specially referred to. The current was applied in each experiment for six successive periods of 10 minutes each, with intervals of five minutes between, and the tube containing the emulsion was kept surrounded by melting ice. Under these conditions the temperature of the emulsion never rose above 32° C., a degree of heat which, for the time duration of the experiments, would not have any injurious effect upon either of the species tested.

Experiment 1.—A thick, deep red emulsion of *B. prodigiosus* in distilled water was exposed to the action of the current. A certain amount of sparking occurred just above the surface of the emulsion from the wire which was connected with the terminal platinum disc. The red colour of the emulsion was quickly discharged, and the bacteria were all killed at the end of the experiment. The emulsion had become strongly acid, 1 c.c. of it containing 0·1 c.c. of N/40 acid, equivalent to 0·016 per cent. of nitric acid.

In a control experiment, carried out without immersion of the tube in melting ice, the temperature of the emulsion had risen to 65° C. at the end of the 60 minutes' exposure.

Experiment 2.—The organism tested was *B. pyocyaneus* in an emulsion with normal saline solution, and a constant stream of hydrogen was passed through the fluid. In this instance, again, the bacteria were all destroyed at the end of the experiment, and the emulsion was found to contain in solution some substance which was capable of liberating iodine from iodide of potassium.

Experiment 3.—An emulsion of *B. pyocyaneus* with normal saline solution was again used; hydrogen was passed through the tube above the level of the

emulsion so as to exclude atmospheric air without causing the constant splashing of the fluid which had occurred in the last experiment. After 60 minutes' exposure, as in the other experiments, to the action of the current, the bacteria were found to be unaffected, the emulsion yielding equally free growth before and after treatment.

A large number of experiments in which the discharge was sprayed on to the emulsion through an atmosphere of some pure gas were also carried out, with appropriate arrangements for the conduction of the current through the emulsion. These experiments will be referred to in detail after the influence of the nitrous compounds which are formed when the discharge occurs in common air have been considered, and meanwhile it may be said that the result of such further experiments was in confirmation of the conclusion at which we had already arrived, that is to say, it appeared as if the discharge and subsequent passage of the current through the emulsion had, of itself, no injurious influence on the bacteria under the time conditions of our experiments.

IV.—The Possible Action of Light Rays Resulting from the Electrical Discharge.

Experiments were carried out in order to ascertain whether the light rays resulting from the electrical discharge might have had any definite influence in the production of the germicidal effects which were observed in our previous experiments. And in the result it may be said that we were able to exclude any such factor as operative under the time conditions of our experiments.

The following experiment bearing on this point may be mentioned. Tubes of melted nutrient agar were cooled down to 41° C., and inoculated severally from cultures of the bacteria with which we had previously experimented; the medium was then poured out into Petri dishes, and allowed to set. The covers of the Petri dishes were thickly coated with black varnish, except for a small circular area which was left unprotected. The Petri dishes were then closely exposed for 60 minutes to the action of light rays from discharges such as those which had been employed in the other experiments, the dishes being so arranged that the maximum effect of the rays would be exercised on the area of inoculated agar immediately underlying the unprotected part of the cover. After exposure the dishes were incubated at temperatures suitable to growth of the respective bacteria, and in every case good growth was obtained over the whole surface of the medium, there being no indication of inhibition of growth in those portions which had been specially exposed to the action of the light rays.

V.—The Influence of Heat Rays Resulting from the Electrical Discharge.

In all our experiments, unless the contrary is expressly stated, means were adopted to prevent the temperature of the bacterial emulsion rising to a point at which the direct germicidal action of heat would come into play. The highest temperature reached, when limiting precautions were adopted, was about 32° C., which was recorded in some experiments which have already been referred to, when the terminal platinum disc was actually immersed in the emulsion; in experiments in which the discharge was sprayed from a platinum brush suspended above the surface of the fluid the maximum temperature reached was usually several degrees below this point.

In order to satisfy ourselves that we were experimenting under safe temperature conditions we tested the resistance to heat of some of the cultures of bacteria which were used in our experiments. We ascertained that the culture of *B. prodigiosus* survived a temperature of 45° C. with an exposure of 60 minutes, but was destroyed by an exposure to 50° C. for 30 minutes; *B. pyocyaneus* survived an exposure to a temperature of 50° C. for 45 minutes, but was killed at the same temperature with an exposure of 60 minutes; the cultures of *B. coli communis* and *Micrococcus pyogenes aureus* were both unaffected by 60 minutes' exposure at a temperature of 50° C.

The results of these tests with regard to temperature and duration of exposure are tabulated below:—

	40° C.	45° C.			50° C.		
	60 mins.	39 mins.	45 mins.	60 mins.	30 mins.	45 mins.	60 mins.
B. prodigiosus	+	+	+	+	0	0	0
B. pyocyaneus	+	+	+	+	+	+	0
B. coli communis ...	+	+	+	+	+	+	+
M. pyogenes aureus	+	+	+	+	+	+	+

But, whilst we were able to eliminate the direct germicidal effect of heat in considering the results of our experiments, it was clear that under certain conditions the germicidal action of the nitrous and nitric compounds and the hydrogen peroxide which were formed as the result of the discharge, and taken into solution in the bacterial emulsion, would be considerably enhanced by a rise of temperature which would not by itself have any direct action on the bacteria; and it is to the varying degree of germicidal energy of these oxidising substances at temperatures between about 15° C. and 35° C. that

apparent discrepancies in some of our results must be partly attributed. For with variation in the amplitude of the electric current, which frequently occurred in the course of our experiments, it was not possible to keep the temperature of the emulsions at a constant point; the most that we succeeded in doing was to ensure by cooling arrangements that the temperature did not rise to a point at which the direct germicidal effect of heat would come into action.

VI.—The Influence, on Bacteria Suspended in Water, of Nitrous and Nitric Acids resulting from the Action of the Electrical Discharge on the Air and taken up in Solution.

We next proceeded to test the germicidal action of sterile distilled water which had been exposed to discharges from the high-frequency apparatus whilst contained in tubes under conditions similar to those which prevailed in the experiments detailed in Table I.

On adding iodide of potassium to distilled water which has been exposed to the action of the discharge for a few minutes, a considerable amount of iodine is set free, and further tests show the presence of nitrous and nitric acids in solution.

The immediate products of the action of the discharge on ordinary air probably include ozone, hydrogen peroxide, nitrogen peroxide, and perhaps other oxides of nitrogen. A certain amount of the substances thus formed are taken up in solution by the water, and the ozone and hydrogen peroxide are probably rapidly decomposed by reaction with the other products present, nitric oxide being converted to nitrogen peroxide, and nitrous to nitric acid.

The nitrogen peroxide which is formed reacts with water to form nitrous and nitric acids according to the equation

$$2NO_2 + H_2O = HNO_2 + HNO_3.$$

Under the conditions of the experiment a part of the nitrous acid decomposes into nitric acid, nitric oxide, and water,

$$3HNO_2 = HNO_3 + 2NO + H_2O,$$

and the nitric oxide would probably again be converted into nitrogen peroxide and nitrous and nitric acids.

Our experiments were carried out as follows:—After each tube had been exposed to the action of the discharge for a given time the resulting acidity was estimated in terms of nitric acid, and the germicidal action of the solution was tested on a single species of bacterium. For the latter purpose two loopfuls of growth from a culture on agar were rubbed up into an

emulsion with the acid solution, and subcultures were made after the organism had been exposed to the action of the acid solution for periods of 15, 30, and 60 minutes. The inoculated tubes were then incubated for 96 hours at an appropriate temperature, and the amount of growth, if any, compared with that obtained from similar subcultures made from similar emulsions in distilled water.

The results of these experiments are given on Table III:—

Table III.

Species.	Duration of the exposure of water to the discharge.	Current at 100 volts supplied to the motor driving the alternator.	Acidity (calculated as nitric acid per cubic centimetre) of solution at end of experiment.	Percentage of acid in emulsion after experiment (calculated as nitric acid).	Result of exposure of bacteria to acid solution for times as below. + = growth obtained. 0 = no growth.		
					15 mins.	30 mins.	60 mins.
Series 1.	mins.	ampères.					
B. typhosus	30	6	0·0022	0·22	0	0	0
B. coli communis ..	30	6	0·0028	0·28	0	0	0
B. prodigiosus ...	30	6	0·0033	0·33	0	0	0
Series 2.							
B. anthracis	15	6	0·001	0·1	+	+	
B. typhosus	15	6	0·0007	0·07	0	0	
B. coli communis ..	15	6	0·0011	0·11	0	0	
B. diphtheriæ......	15	6	0·0008	0·08	0	0	
B. pyocyaneus ...	15	6	0·0013	0·13	0	0	
B. prodigiosus ...	15	6	0·00097	0·097	0	0	

From the results shown in Table III it will be seen that, except as regards the tube in which a sporing culture of *B. anthracis* was treated, the substances taken up from the air by water exposed to the action of the discharge were sufficient to destroy the bacteria subsequently immersed in the acid solution for so short a period as 15 minutes. It will be seen also that, although the tubes used in each series of experiments were exposed to the action of the discharge under conditions as nearly equal as the apparatus and current at our disposal would allow, there was considerable variation in the resulting degree of acidity, as had occurred also in the experiments detailed in Tables I and II. In short, it soon became obvious that we were utilising forces which were not under nice control, and that our results must be taken as being comparative in only an approximate degree.

The extent to which discrepancy in the degree of acid produced occurred, under conditions as nearly equal with regard to current as we could obtain, is shown in the following tabulation of the results of the acidimetry of the contents of each tube after exposure. For purposes of comparison the experiments of Tables I and III may be considered together, as having been carried out with the same apparatus, whilst in the experiments of Table II a different arrangement of electrical apparatus was used, and one which was less suitable to the purposes of our experiments (see p. 66).

Acidimetry Table.

	Current at 100 volts supplied to motor driving the alternator.	Duration of exposure to discharge in mins.	Number of tubes used.	Percentage average acidity, calculated as nitric acid.	Percentage acidity of contents of each tube, calculated as nitric acid.
Table I.					
Series 1.........	7 ampères	15	6	0·216	0·24, 0·23, 0·23, 0·21, 0·2, 0·19
Series 2.........	6·75 „	10	6	0·141	0·16, 0·14, 0·14, 0·14, 0·14, 0·13
Series 3.........	6 „	10	4	0·097	0·12, 0·11, 0·08, 0·08
„	9 „	15	1	—	0·25
„	9 „	7·5	1	—	0·09
Table III.					
Series 1.........	6 „	30	3	0·276	0·33, 0·28, 0·22
Series 2.........	6 „	15	6	0·099	0·13, 0·11, 0·1, 0·097, 0·08, 0·07
	Current and voltage (primary coil).				
Table II.					
Series 1.........	4 ampères 74 volts	20	6	0·053	0·09, 0·09, 0·05, 0·04, 0·04, 0·01
Series 2.........	6 ampères 80 volts	15	6	0·061	0·1, 0·07, 0·06, 0·05, 0·05, 0·04
Series 3.........	6 ampères 84 volts	10	3	0·063	0·08, 0·06, 0·05

We are unable to explain satisfactorily the unequal formation of acid in tubes of distilled water, or of bacterial emulsion, which have been submitted to apparently the same treatment in the course of each separate series of experiments, we can only assume that it is a result of considerable variation in the intensity of the current during the progress of an experiment. The results obtained in the experiments of Table III show, at any rate, that a natural solution of the products of the action of the discharge on common

air is sufficient when these products are present in such quantity that the acidity of the fluid is equivalent to 0·07 per cent. of nitric acid to destroy a non-sporing bacillus, such as *B. typhosus*, after it has been exposed to the action of the solution for 15 minutes. And we take it that in the cases in which a bacterial emulsion after sterilisation by exposure to the discharge is found to present an acidity in excess of 0·07 per cent. the sterilisation may be fairly attributed mainly to these chemical products, in the absence of any indication that other actively germicidal influences have been at work.

We may, however, point out that the experiments of Table III are not absolutely comparable with those of Table I, since in the latter set of experiments the quantity of germicidal substances in solution gradually increased up to the end of the exposure, when the estimation gives only the total acidity at the end of the experiment, whereas in the experiments of Table III the bacteria were immersed in a solution of the maximum acidity for the full period of the experiment.

On the other hand, the germicidal action of such substances as are present is probably exercised more energetically under the conditions prevailing in the experiments of Table I, when the bacteria are immersed in the fluid during the actual formation and solution of the nitrous and nitric bodies and are so exposed to the action of these substances whilst still in a nascent condition, than it is under the conditions prevailing in the experiments of Table III, when the bacteria are immersed in a fluid in which the chemical changes are for the most part finished, except for the comparatively slow oxidation of nitrous to nitric acid. Moreover, in the experiments of Table I there would be a continuous molecular disturbance of the fluid which would probably assist the action of chemical germicides.

Although other substances are formed in the air, and doubtless taken up in solution by the water, in addition to the nitrous and nitric compounds, it was to these latter that we attributed chiefly the germicidal action, and it was these that we considered as the causes of the acidity of the solution. We next prepared artificial mixtures of nitrous and nitric acid with a total acidity approximating to that obtained in some of our experiments, and then tested the germicidal action of the solutions. Our solutions were prepared by dissolving potassium nitrite in water and adding certain quantities of nitric acid.

The actual composition of the several solutions was as follows:—

Solution.	Weight of potassium nitrite in 1 c.c.	Equivalent weight of nitrous acid in 1 c.c.	Weight of nitric acid in 1 c.c.
	gramme.	gramme.	gramme.
a	0·00074	0·00041	0·0005
b	0·00148	0·00082	0·001
c	0·00222	0·00123	0·0015
d	0·00296	0·00164	0·002
e	0·0037	0·00205	0·0025

Assuming that all the available nitrous acid is liberated by the nitric acid added, the composition of each solution at the time of carrying out the experiment would be—

Solution.	Weight of nitrous acid in 1 c.c.	Weight of nitric acid in 1 c.c.	Weight of potassium nitrate in 1 c.c.
	gramme.	gramme.	gramme.
a	0·00041	0·00009	0·00088
b	0·00082	0·00018	0·00176
c	0·00123	0·00027	0·00264
d	0·00164	0·00036	0·00352
e	0·00205	0·00045	0·0044

But whatever the interaction between the nitrite and the nitric acid may be, one may assume that the acidity of the respective solutions was 0·05, 0·1, 0·15, 0·2 and 0·25 per cent. respectively, calculated as nitric acid.

Nitrous acid being unstable, and decomposing readily at ordinary temperatures into nitric acid, nitric oxide, and water, we carried out two sets of experiments, one at a temperature of 0° C., the other at a temperature of 15° C.

Immediately after they had been prepared the respective solutions were inoculated with two loopfuls of the several bateria tested: loopfuls of the acid emulsion were taken immediately after the bacteria had been introduced ("momentary" exposure), after an interval of 15 minutes, and after an interval of 30 minutes nutrient agar tubes were inoculated with the loopfuls and incubated side by side with control tubes.

The results of the experiments are given in Table IV.

Table IV.

Acidity of solution estimated in terms of nitric acid.	Temperature at which experiment was carried out.	*B. anthracis* (sporing culture).			*B. typhosus.*			*B. pyocyaneus.*			*B. prodigiosus.*		
		Duration of exposure.			Duration of exposure.			Duration of exposure.			Duration of exposure.		
		"Momentary."	15 minutes.	30 minutes.	"Momentary."	15 minutes.	30 minutes.	"Momentary."	15 minutes.	30 minutes.	"Momentary."	15 minutes.	30 minutes.
	° C.												
a. 0·05 p.c. ...	0	+	+	+	+	0	0	+	0	0	+	Very scanty	0
	15	+	0	0	0	0	0	+	0	0	+	0	0
b. 0·1 p.c. ...	0	+	+	+	+	0	0	0	0	0	+	0	0
	15	+	0	0	0	0	0	0	0	0	+	0	0
c. 0·15 p.c. ...	0	+	+	+	+	0	0	0	0	0	0	0	0
	15	+	0	0	0	0	0	0	0	0	0	0	0
d. 0·2 p.c. ...	0	+	+	+	Very scanty	0	0	0	0	0	0	0	0
	15	+	0	0	0	0	0	0	0	0	0	0	0
e. 0·25 p.c. ...	0	+	+	0	0	0	0	0	0	0	0	0	0
	15	+	0	0	0	0	0	0	0	0	0	0	0

VII.—As to the Action of Ozone and Hydrogen Peroxide Formed in the Air as the Result of the Electrical Discharge.

Ozone and hydrogen peroxide had to be considered amongst the products resulting from electrical discharge in an atmosphere of common air and in the presence of aqueous vapour, for certain quantities of these substances would doubtless be taken up in solution by the bacterial emulsion.

But under the conditions of the experiments, that is to say with a simultaneous formation of nitrous acid, it is, we think, quite certain that any quantity of ozone or hydrogen peroxide which passed into solution would be immediately decomposed. And, therefore, the importance of these two substances in these experiments probably does not lie in any relation to their germicidal action, but rather in the part which they play in the oxidation of the nitrous compounds.

Peroxide of hydrogen, however, became of direct importance in some experiments in which the electrical discharge was effected in an atmosphere

of pure hydrogen, for in this case it appeared as if this substance were the active germicidal agent in certain cases which will be referred to in detail further on.

VIII.—Results of Experiments in which the Electrical Discharge was Sprayed Through an Atmosphere of Various Pure Gases on to Bacteria Suspended in Water.

The remaining experiments which we have to describe were carried out by spraying the electrical discharge on to the bacterial emulsion through an atmosphere of one or other of the following pure gases:—Hydrogen, carbon dioxide, carbon monoxide, and nitrogen. The object of these experiments was, by discharging the current under conditions such that electrolytic changes were likely to be reduced to a minimum, to observe the action of the discharge and current on the bacteria without the concomitant germicidal action of the nitrous and nitric acids which are formed under the ordinary conditions when the discharges are used in medical practice. In some instances we succeeded in our object, inasmuch as we found that when electrolytic changes were inappreciable, or nearly so, we were able to expose the bacteria to the action of the discharge for periods of 30 and 60 minutes without affecting their subsequent growth in any way. But in many other experiments we found that, even when the discharge occurred in an atmosphere of a pure gas, electrolytic changes resulted after a time, in most cases probably depending upon the rising of water vapour in the tube from the emulsion with the formation of peroxide of hydrogen and possibly other germicidal substances.

Experiments in an Atmosphere of Pure Hydrogen.

The hydrogen used in our experiments was prepared by the action of diluted pure sulphuric acid on pure zinc, the gas being passed successively through solutions of potassium hydroxide, potassium permanganate, and silver nitrate.

The gas thus prepared had no appreciable action on bacteria under the time conditions of our experiments; it was passed continuously for 90 minutes through bacterial emulsions such as those used in our subsequent experiments, and sub-cultures made from the emulsion after the passage showed that the gas had no injurious action.

A few preliminary experiments were carried out with emulsions of *B. pyocyaneus* and *B. prodigiosus*, the current being discharged from a brush of twisted platinum wire suspended in an atmosphere of hydrogen 2·5 cm. above the emulsion. The results are given in Table V:—

Table V.

Species.	Duration of exposure to the discharge.	Current at 100 volts supplied to motor driving the alternator.	Reaction of the emulsion at the end of the exposure.	Result of sub-culture from the emulsion after the exposure.
	mins.	ampères.		
B. pyocyaneus......	30	5·8	Neutral	Good growth, pigment formation normal
	60	5·8	"	" "
B. prodigiosus......	30	5·0	"	" "
	60	5·0	"	" "

In these experiments there was no appreciable change in the reaction of the emulsion after exposure to the discharge; in a set of control experiments carried out with exactly the same arrangement of apparatus, but with the discharge in an atmosphere of common air, the emulsion became strongly acid from the formation of nitrous and nitric acids, and the bacteria were destroyed at the end of the 30 minutes' exposure.

In other experiments, in which the action of the discharge in an atmosphere of hydrogen was tested, distinct germicidal effects were observed in some instances. In these experiments a more energetic discharge was obtained by the use of the platinum disc brush with nine points of platinum wire; in one series of experiments the current was obtained from the alternator, in one other series by means of the Ruhmkoff coil, and the hydrogen was passed continuously through the emulsion during its exposure, with splashing of the emulsion as a consequence. The results are given in Table VI, some of the tests being carried out in duplicate.

It will be seen that in a number of the experiments detailed in Table VI the bacteria were destroyed; with the current from the alternator *B. typhosus* was destroyed after an exposure of 60 minutes, having previously survived an exposure of 30 minutes, and *Micrococcus agilis* was destroyed after an exposure of 30 minutes, whilst all the other bacteria survived. With the use of the current from the Ruhmkoff coil germicidal action was more frequent; *B. typhosus* was destroyed after an exposure of 30 minutes, *B. dysenteriæ* was destroyed after an exposure of 30 minutes in one instance, but yielded a scanty growth in a duplicate experiment; *B. coli communis* was destroyed after an exposure of 30 minutes in one experiment, but survived an exposure of 60 minutes in another; *B. pyocyaneus* was destroyed after an exposure of 30 minutes in duplicated experiments, and *Micrococcus agilis* was destroyed after an exposure of 30 minutes.

Table VI.

Species.	Duration of exposure.	Experiments with current at 100 volts (5 ampères) supplied to motor driving the alternator.		Experiments using Ruhmkoff coil.	
		Reaction of emulsion at end of exposure.	Result.	Reaction of emulsion at end of exposure.	Result.
	mins.				
B. anthracis …………	30	—	—	Neutral …………	+
	60	Neutral …………	+		
	60	,, …………	+		
B. typhosus …………	30	,, …………	+	,, …………	0
	30	,, …………	+		
	60	,, …………	0		
	60	Faintly alkaline	0		
B. dysenteriæ (Shiga)	30	—	—	,, …………	Scanty growth
	30	—	—	,, …………	0
B. coli communis ……	30	Neutral …………	+	,, …………	0
	60	—	—	,, …………	+
B. pyocyaneus ………	30	Neutral …………	+	,, …………	0
	30	,, …………	+	,, …………	0
	60	,, …………	+		
	60	Faintly alkaline	+		
B. prodigiosus ………	30	Neutral …………	+		
	30	,, …………	+		
	60	,, …………	+		
	60	,, …………	+		
Micrococcus agilis …	30	,, …………	0	Neutral …………	0

In two of the experiments the reaction of the emulsion was found to be faintly alkaline at the end of the exposure, one experiment being that in which *B. typhosus* was destroyed after exposure to the current from the alternator for 60 minutes, and the other one of the instances in which *B. pyocyaneus* survived exposure to the same current for 60 minutes. In all the other experiments, whether the current was obtained from the alternator or from the Ruhmkoff coil, the reaction of the emulsion remained practically neutral after the exposure.

In the experiments of Table V the atmosphere of hydrogen was obtained by passing the gas into the tube above the surface of the emulsion; in the other experiments the gas was passed through the emulsion with resulting disturbance of the liquid and increased opportunity for electrolytic changes to occur, and, moreover, in the experiments of Table VI a somewhat more energetic discharge was obtained from the disc brush than from the twisted wire brush used in the earlier experiments. We had, therefore, to consider whether the germicidal effect which had been produced in some of the

experiments carried out under the conditions more favourable to electrolytic changes might have been caused by the known possible substances which might be produced by the action of an electrical discharge on hydrogen in the presence of water (hydrogen oxide) vapour.

The substances which theoretically might be thus produced are ozone, nascent oxygen, nascent hydrogen, and hydrogen peroxide, and we proceeded to try whether we could detect either ozone or hydrogen peroxide after discharging the current in an atmosphere of pure hydrogen over water, the discharges being continued for periods of 15, 30, and 60 minutes. In each case peroxide of hydrogen could be detected in the water after exposure to the discharge, and a piece of moist starch iodide paper introduced into the tube at the termination of each time exposure gave no indication of the presence of ozone.

We next carried out two sets of experiments in order to ascertain how far the germicidal effect produced in some of the previous experiments could be attributed to the action of chemical substances, of which hydrogen peroxide was at any rate an important one, taken up in solution by the emulsion.

In each experiment 25 c.c. of distilled water in a test-tube were exposed to the action of the discharge for 60 minutes: in the first set of experiments pure hydrogen was passed through the water in order to expel any dissolved oxygen or nitrogen before exposure to the discharge commenced, and was then passed into the tube above the level of the water for the 60 minutes' exposure; in the second set of experiments a stream of hydrogen was passed continuously through the water during the whole exposure. After exposure to the discharge 5 c.c. of the treated water were titrated with sodium thiosulphate and starch for the quantitative estimation of the peroxide of hydrogen, the presence of which was indicated by the various tests applicable. Quantities of 5 c.c. were then pipetted off into test-tubes, and inoculated respectively with two loopfuls of cultures of *B. anthracis*, *B. coli communis*, and *B. typhosus* from agar tubes. Loopfuls of the bacterial emulsion were transplanted to agar tubes at intervals of 15, 30, and 60 minutes, and incubated in the usual way to test the vitality of the bacteria after their immersion in the solution of hydrogen peroxide.

The results of these two sets of experiments are given in Table VII.

Table VII.

Cultures taken from the bacterial emulsion after	Quantity of peroxide of hydrogen in solution after exposure to the discharge for 60 minutes.					
	I.—One part in 15,000 (approximately).			II.—One part in 10,000.		
	15 mins.	30 mins.	60 mins.	15 mins.	30 mins.	60 mins.
B. anthracis	+	+	+	+	+	+
B. coli communis	+	+	+	+	+	0
B. typhosus	+	+	+	0	0	0

The results of these last experiments showed that when the discharge was sparked on to distilled water through an atmosphere of hydrogen under conditions similar to those prevailing in the experiments of Table V, except that the platinum disc brush was used instead of the twisted wire brush, peroxide of hydrogen was taken up in solution to the amount of 1 in 15,000, and bacteria, which were subsequently immersed in the solution for as long as 60 minutes, were not apparently affected in any way. But when the discharge was sparked on to the water whilst hydrogen was passing continuously through it, under conditions similar to those prevailing in the experiments of Table VI, the amount of hydrogen peroxide taken up in solution increased to 1 in 10,000, and *B. typhosus* and *B. coli communis* were destroyed after immersion in the solution for 15 and 60 minutes respectively. We were unable to detect any change in the chemical composition of the water other than that due to the presence of peroxide of hydrogen, and it was to the presence of this substance that we attributed the germicidal action of the solution.

In comparing the results of the experiments of Tables VI and VII it must be remembered that, as in the similar experiments with nitrous compounds, the germicidal action of peroxide of hydrogen is most active when the substance is in the nascent condition. Thus Bonjean* found that the nascent hydrogen peroxide liberated from calcium peroxide completely sterilised the notoriously foul Seine water in four hours when acting in a dilution of 1 in 14,285, whilst a dilution of 1 in 3445, when obtained by means of a commercial solution of hydrogen peroxide, did not sterilise the same polluted water until it had acted for six hours. In the experiments of Table VI,

* 'Comptes Rendus de l'Académie des Sciences,' vol. 140, p. 50, 1905.

moreover, it appeared probable that in addition to the action of peroxide of hydrogen there might be another factor at work, for a consideration of the chemical decomposition by which the peroxide was produced suggested the simultaneous liberation of quantities of nascent hydrogen which would doubtless also exercise a powerful germicidal action.

Assuming, however, that the germicidal action of peroxide of hydrogen was the main factor concerned, we found that some experiments which we carried out to test the comparative resistance of the bacteria used in the experiments to the action of the peroxide gave results which were roughly in accordance with those detailed in Table VI. We found that sporing cultures of *B. anthracis* were strongly resistant; experiments carried out at temperatures of 0° C., 15° C., and 26° C. with a 1-per-cent. solution of peroxide of hydrogen showed that with 30 minutes' immersion no obvious effect on the bacteria was produced at any of the temperatures, with 60 minutes' immersion no effect was produced at a temperature of 15° C., but there was distinct inhibition of subsequent growth in experiments carried out at 26° C., whilst the bacteria were destroyed after 120 minutes' immersion at all three temperatures. With regard to the non-sporing bacilli, we found that *B. prodigiosus* and *B. pyocyaneus* were more resistant to the peroxide than *B. coli communis*, *B. dysenteriæ*, and *B. typhosus*, the last mentioned being readily destroyed by very dilute solutions, and *B. prodigiosus* exhibiting a marked degree of resistance. In the performance of these experiments it was noted that the rapidity of the decomposition of the peroxide in the solution, and the consequent activity of germicidal action, appeared to depend to some extent upon individual peculiarities in the chemical constitution of the various species used in the tests. The experiments were carried out by introducing definite quantities of cultures of each species from nutrient agar into test-tubes containing the diluted peroxide and kept at the several temperatures. And it was apparent that the rapidity with which the peroxide was decomposed, as measured by the evolution of oxygen in bubbles from the solution, varied widely with different species at the same temperature; for any one species the evolution of gas was more rapid at the higher temperature, with corresponding intensity of germicidal action.

In these experiments to test the germicidal action of solutions of peroxide of hydrogen, a special effect of the peroxide in temporarily inhibiting the formation of pigment by chromogenic bacteria which had been immersed in solutions of it was also noticed. Thus, after an immersion for 60 minutes in a 1 in 500 solution of peroxide of hydrogen kept at a temperature of either 15° C. or 26° C. the amount of subsequent growth of *B. prodigiosus* was in no way affected; but whereas in control cultures of the bacillus which had not

been exposed to the action of the peroxide the formation of pigment was obvious from the first appearance of growth, in the cultures from bacilli which had been immersed in the solution the formation of pigment was not apparent until the third or fourth day of incubation. This effect was repeatedly noticed in experiments with this organism, and also with the other chromogenic organism tested, *B. pyocyaneus.*

Experiments in an Atmosphere of Pure Carbon Dioxide.

We next tested the action of the discharges when sprayed on to bacterial emulsions through an atmosphere of pure carbon dioxide, the gas having been prepared by the action of hydrochloric acid on marble and purified by passage through a solution of nitrate of silver.

Experiments which have been recorded by Dr. Charles Slater* showed that it was improbable that under the time conditions of our experiments carbon dioxide itself would have any action on the bacteria; but we first tested the action of the gas by passing it rapidly through bacterial emulsions for periods of 60 minutes, and found that no degree of germicidal action was manifested.

The results of a few experiments in which the discharge was sprayed on to the bacterial emulsion through an atmosphere of carbon dioxide are given in Table VIII.

Table VIII.

Species.	Current at 100 volts supplied to motor driving the alternator.	Duration of exposure to the discharge.	Result.
	ampères.	mins.	
B. typhosus	5	15	+
	6	30	0
	5	45	Scanty growth.
	5	60	0
	5	60	0
B. pyocyaneus	—	60	0
		60	0
B. prodigiosus	—	15	+
		30	+
		60	0
		60	0

When the discharge is sprayed on to the bacterial emulsion through an atmosphere of carbon dioxide, distinct germicidal effects are obtained in some experiments; but, as was the case when the discharge occurred in an atmosphere of hydrogen, we found that peroxide of hydrogen was taken up in

* 'Journal of Pathology and Bacteriology,' vol. 1, p. 468 1893.

solution in considerable quantities after the exposure of the emulsion for as short a period as 15 minutes. It is probable that under the action of the discharge carbon dioxide is decomposed into carbon monoxide, oxygen,* and possibly other products, and the hydrogen peroxide is then formed by the action of the oxygen in nascent condition on the water vapour present. Some other substance, which rapidly liberated iodine from iodide of potassium, was also present in the solution, but starch iodide papers suspended in the tubes above the emulsion gave no indication of the presence of ozone.

It is perhaps worth mentioning that the colour of the discharge spark in an atmosphere of carbon dioxide alters in appearance after the experiment has been in progress for a few minutes. At first the spark presents a peculiar green colour, but after a few minutes the appearance alters to that of a combination of green with a very pale violet colour, so that often whilst the upper part of the spark is of a distinct green colour the lower part appears to spread out in the form of a fine network of violet rays.

Experiments in an Atmosphere of Carbon Monoxide.

One series of experiments in which the discharge from the alternator was sprayed through an atmosphere of carbon monoxide on to emulsions containing *B. pyocyaneus* and *B. prodigiosus* respectively was carried out.

The carbon monoxide was prepared by acting on sodium formate with concentrated sulphuric acid, and the gas was passed slowly from the gasholder to another through tubes of the emulsions as they were being subjected to the action of the discharge. In every experiment of this series the non-sporing bacteria were destroyed after an exposure to the discharge for 30 minutes, the current supplied to the motor driving the alternator being 5 ampères, and the potential difference being 100 volts. In all these experiments, again, peroxide of hydrogen was found to be present in some quantity after the emulsion had been exposed to the action of the discharge for as short a period as 10 minutes. According to Berthelot,† carbon dioxide and other products are formed when a silent electrical discharge is passed through carbon monoxide.

Experiments in an Atmosphere of Nitrogen.

Two series of experiments in which *B. pyocyaneus* and *B. prodigiosus* were exposed to the action of the discharge in an atmosphere of nitrogen were

* Compare results obtained by Collie on sparking carbon dioxide in vacuum tubes ('Chem. Soc. Trans.,' 1901, p. 1063). Also compare Berthelot ('Compt. Rend.,' 1898, vol. 126).

† *Loc. cit.*

carried out. In one series of experiments the gas, probably mixed with argon, etc., was obtained from common air by passing it over heated copper; in the other series the gas was prepared by heating a mixture of strong solutions of potassium nitrite and ammonium chloride, the gas being subsequently purified by passage through strong sulphuric acid. And in every experiment carried out under these conditions the bacteria were destroyed after exposure to the discharge for 30 minutes, the current being of amplitude similar to that used for the experiments in carbon monoxide.

After exposure to the discharge the emulsions were found to be strongly acid in reaction and to contain quantities of nitrous and nitric acids which had probably been formed by the action of the discharge on nitrogen in the presence of water vapour.

IX.—Conclusions.

The results of our experiments may be summarised as follows:—

1. When bacteria suspended in water are exposed in an atmosphere of common air to the action of electrical discharges of high potential and rapid frequency, such as are used in medicine for purposes of treatment, sufficient quantities of nitrous and nitric acids are taken up in solution within as short a period as 15 minutes to sterilise the emulsion, and the germicidal action of these compounds in their nascent condition is favoured under ordinary circumstances (*a*) by the heating of the medium in which they are suspended by heat rays resulting from the discharge, and (*b*) by the concomitant formation of substances such as ozone and peroxide of hydrogen, which, readily yielding up a portion of their oxygen, accelerate the interaction of nitrous and nitric acids and their consequent germicidal activity.

2. When the bacteria are exposed to the action of the discharge in an atmosphere of pure hydrogen, under similar conditions, there is a decomposition of the water vapour necessarily present in the atmosphere of the tube with the formation of peroxide of hydrogen in quantities such as are sufficient after a time to exercise distinct germicidal action in the case of some bacteria.

3. When exposure to the discharge occurs in atmospheres of pure carbon dioxide or carbon monoxide there may again be sufficient peroxide of hydrogen formed to exercise germicidal action.

4. When exposure to the discharge occurs in an atmosphere of pure nitrogen, sterilisation may be effected by the action of nitrous and nitric acids.

5. The action on bacteria of the light rays resulting from the discharge is negligible under the time conditions of our experiments.

6. In all cases in which germicidal action was manifest it appeared to us that the result was due to the action of chemical substances formed by the discharge, either from the surrounding atmosphere or from the water in which the bacteria were suspended; and in no case did we obtain any evidence that, under the time conditions, the electrical current or its discharge had any direct injurious influence on the bacteria, apart from the accompanying formation of chemical germicidal substances and from whatever effect may be exercised by the heat rays.

In considering the application of the results of our experiments to the explanation of the results which are obtained in medical practice by the use of high-frequency discharges in the treatment of lupus and other diseases in which surface ulceration has occurred, the different conditions under which bacteria are exposed to the action of the discharge must be taken into account.

In our experiments the bacteria were immersed in a column of water of about 3 cm. depth, but the water would probably be in a state of continuous molecular disturbance, which would tend to bring the bacteria into intimate contact with the chemical substances which are being taken up in solution during the exposure.

In cases of lupus and the various conditions of ulceration in which the high-frequency discharges are used in medical practice the bacteria are either exposed in a film of albuminous fluid on the surface under treatment, or may be more or less deeply embedded in granulation tissue. When bacteria are exposed on the surface it is obvious that the germicidal action of nitrous and nitric acids formed in the air as the result of the discharge will be readily and effectually exercised; and even when the bacteria are embedded in granulation tissue at a slight depth from the surface we think that it is possible that some penetration of the tissue by these substances in a nascent and active condition may occur, favoured doubtless by the "impact" action of the discharge. And, in any case, we feel justified in assuming as the result of our experiments that under the time conditions which prevail in the use of these discharges in medical practice the electric current itself has no special or direct injurious influence on the bacteria present, and that, therapeutically, treatment by the use of these discharges under the given conditions must be regarded merely as an efficient means for the intimate application of chemical germicides formed from the atmosphere in which the discharge takes place. Under the conditions existing in practice the heat rays resulting from the discharge also probably assist in the germicidal action in some degree.

The use of these discharges in conditions of disease, such as carcinoma and

sarcoma, not due to bacterial infections, has not come within the scope of our present inquiry, but we may say that we think it probable that when the discharge is sprayed on to the unbroken skin there may be some local absorption of nitrous compounds under the influence of the discharge, and that these may have some effect upon cells immediately underlying the area of skin to which the discharges are applied. The "skinning over" of the ulcerated surface of malignant new growths, without much, or any, effect upon the underlying new formation, which has occasionally been observed, may be attributed to the chemical germicidal action affecting the bacteria to which the ulceration is due.

We have to express our thanks to Dr. C. R. C. Lyster, the Medical Officer in charge of the Electrical Department at the Middlesex Hospital, for his kindness in placing the electrical apparatus at our disposal for these experiments.